This report is required by law (7 USC 2143). Failure to report according to the regulations can result in an order to cease and desist and to be subject to penalties as provided for in Section 2150.

See reverse side for additional information.

Interagency Report Control No 0180-DOA-AN

UNITED STATES DEPARTMENT OF AGRICULTURE
ANIMAL AND PLANT HEALTH INSPECTION SERVICE

ANNUAL REPORT OF RESEARCH FACILITY

(TYPE OR PRINT)

1. REGISTRATION NO. 58-R-0003 **CUSTOMER NO.** 859

FORM APPROVED OMB NO. 0579-0036

2. HEADQUARTERS RESEARCH FACILITY *(Name and Address, as registered with USDA, include Zip Code)*

UNIVERSITY OF FLORIDA
PO Box 115500
GAINESVILLE, FL. 32611
(352) 392-2978

3. REPORTING FACILITY (List all locations where animals were housed or used in actual research, testing, teaching, or experimentation, or held for these purposes. Attach additional sheets if necessary.)

FACILITY LOCATIONS*(sites)*

See Attached Listing

REPORT OF ANIMALS USED BY OR UNDER CONTROL OF RESEARCH FACILITY *(Attach additional sheets if necessary or use APHIS FORM 7023A)*

A. Animals Covered By The Animal Welfare Regulations	B. Number of animals being bred, conditioned, or held for use in teaching, testing, experiments, research, or surgery but not yet used for such purposes.	C. Number of animals upon which teaching, research, experiments, or tests were conducted involving no pain, distress, or use of pain-relieving drugs.	D. Number of animals upon which experiments, teaching, research, surgery, or tests were conducted involving accompanying pain or distress to the animals and for which appropriate anesthetic, analgesic, or tranquilizing drugs were used.	E. Number of animals upon which teaching, experiments, research, surgery or tests were conducted involving accompanying pain or distress to the animals and for which the use of appropriate anesthetic,analgesic, or tranquilizing drugs would have adversely affected the procedures, results, or interpretation of the teaching, research, experiments, surgery, or tests. *(An explanation of the procedures producing pain or distress in these animals and the reasons such drugs were not used must be attached to this report)*	F. TOTAL NO. OF ANIMALS (Cols. C + D + E)
4. Dogs	6	127	111	0	244
5. Cats	0	34	309	0	343
6. Guinea Pigs	0	0	86	0	86
7. Hamsters	0	0	0	0	0
8. Rabbits	0	14	86	52 4=E520; 30=D290; 18=D968	152
9. Non-Human Primates	13	0	64	0	77
10. Sheep	0	0	113	0	113
11. Pigs	0	16	78	0	94
12. Other Farm Animals					
Equine	0	161	107	0	268
13. Other Animals					
Cattle	0	468	5	0	473
Chinchillas	0	0	40	0	40
Deer Mice	0	483	74	0	557

ASSURANCE STATEMENTS

1) Professionally acceptable standards governing the care, treatment, and use of animals, including appropriate use of anesthetic, analgesic, and tranquilizing drugs, prior to, during, and following actual research, teaching, testing, surgery, or experimentation were followed by this research facility.

2) Each principal investigator has considered alternatives to painful procedures.

3) This facility is adhering to the standards and regulations under the Act, and it has required that exceptions to the standards and regulations be specified and explained by the principal investigator and approved by the Institutional Animal Care and Use Committee (IACUC). **A summary of all the exceptions is attached to this annual report.** In addition to identifying the IACUC-approved exceptions, this summary includes a brief explanation of the exceptions, as well as the species and number of animals affected.

4) The attending veterinarian for this research facility has appropriate authority to ensure the provision of adequate veterinary care and to oversee the adequacy of other aspects of animal care and use.

CERTIFICATION BY HEADQUARTERS RESEARCH FACILITY OFFICIAL
(Chief Executive Officer or Legally Responsible Institutional official)
I certify that the above is true, correct, and complete (7 U.S.C. Section 2143)

(B)(6) (B)(7)(c)

DATE SIGNED 11/29/07

APHIS FORM 7023 (AUG 91) (Replaces VS FORM 18-23 (Oct 88), which is obsolete

PART 1 - HEADQUARTERS

This report is required by law (7 USC 2143). Failure to report according to the regulations can result in an order to cease and desist and to be subject to penalties as provided for in Section 2150.

See reverse side for additional information.

Interagency Report Control No 0180-DOA-AN

UNITED STATES DEPARTMENT OF AGRICULTURE
ANIMAL AND PLANT HEALTH INSPECTION SERVICE

CONTINUATION SHEET FOR ANNUAL REPORT OF RESEARCH FACILITY

(TYPE OR PRINT)

1. REGISTRATION NO. 58-R-0003 **CUSTOMER NO.** 859

FORM APPROVED
OMB NO. 0579-0036

2. HEADQUARTERS RESEARCH FACILITY *(Name and Address, as registered with USDA, include Zip Code)*

UNIVERSITY OF FLORIDA
P.O. Box 115500
GAINESVILLE, FL 32611
(352) 392-2978

REPORT OF ANIMALS USED BY OR UNDER CONTROL OF RESEARCH FACILITY *(Attach additional sheets if necessary or use this form.)*

A. Animals Covered By The Animal Welfare Regulations	B. Number of animals being bred, conditioned, or held for use in teaching, testing, experiments, research, or surgery but not yet used for such purposes.	C. Number of animals upon which teaching, research, experiments, or tests were conducted involving no pain, distress, or use of pain-relieving drugs.	D. Number of animals upon which experiments, teaching, research, surgery, or tests were conducted involving accompanying pain or distress to the animals and for which appropriate anesthetic, analgesic, or tranquilizing drugs were used.	E. Number of animals upon which teaching, experiments, research, surgery or tests were conducted involving accompanying pain or distress to the animals and for which the use of appropriate anesthetic,analgesic, or tranquilizing drugs would have adversely affected the procedures, results, or interpretation of the teaching, research, experiments, surgery, or tests. *(An explanation of the procedures producing pain or distress in these animals and the reasons such drugs were not used must be attached to this report)*	F. TOTAL NO. OF ANIMALS **(Cols. C + D + E)**
Goats	0	30	19	0	49
Singing Mice	0	91	27	0	118
Voles	0	246	0	0	246

ASSURANCE STATEMENTS

1) Professionally acceptable standards governing the care, treatment, and use of animals, including appropriate use of anesthetic, analgesic, and tranquilizing drugs, prior to, during, and following actual research, teaching, testing, surgery, or experimentation were followed by this research facility.

2) Each principal investigator has considered alternatives to painful procedures.

3) This facility is adhering to the standards and regulations under the Act, and it has required that exceptions to the standards and regulations be specified and explained by the principal investigator and approved by the Institutional Animal Care and Use Committee (IACUC). **A summary of all the exceptions is attached to this annual report.** In addition to identifying the IACUC-approved exceptions, this summary includes a brief explanation of the exceptions, as well as the species and number of animals affected.

4) The attending veterinarian for this research facility has appropriate authority to ensure the provision of adequate veterinary care and to oversee the adequacy of other aspects of animal care and use.

CERTIFICATION BY HEADQUARTERS RESEARCH FACILITY OFFICIAL
(Chief Executive Officer or Legally Responsible Institutional official)
I certify that the above is true, correct, and complete (7 U.S.C. Section 2143)

(B)(6) (B)(7)(c)

DATE SIGNED 11/29/07

APHIS FORM 7023A (AUG 91)

(Replaces VS FORM 18-23 (Oct 88), which is obsolete

PART 1 - HEADQUARTERS

Column E Explanation

This form is intended as an aid to completing the Column E explanation. It is not an official form and its use is voluntary. Names, addresses, protocols, veterinary care programs, and the like, are not required as part of an explanation. A Column E explanation must be written so as to be understood by lay persons as well as scientists.

1. Registration Number: 58-R-0003

2. Number 4 of animals used in this study

3. Species (common name) Rabbits of animals used in the study.

4. Explain the procedure producing pain and/or distress.

   Title: E520; Gene regulation of mammalian DNA viruses: Antiviral activity of Imatinib (Gleevec) against Orthopoxviruses

   Please see attached for procedure information.

5. Provide scientific justification why pain and/or distress could not be relieved. State methods or means used to determine the pain and/or distress relief would interfere with test results. (For Federally mandated testing, see Item 6 below)

   Please see attached.

6. What, if any, federal regulations require this procedure? Cite the agency, the code of Federal Regulations (CFR) title number and the specific section number (e.g., APHIS, 9 CFR 113.102):

Agency _____ CFR _____

E520; *Gene regulation of mammalian DNA viruses: Antiviral activity of Imatinib (Gleevec) against Orthopoxviruses*

4. Explain the procedure producing pain and/or distress, including reason(s) for species selected.

Rabbits infected with rabbit pox virus are a unique animal model for smallpox studies because the disease closely mimics the pathology of the human disease, and because rabbit pox virus can be spread between animals in the absence of mechanical or insect vectors. In addition, federal regulations require the use of 2 animal species when using animal data to establish efficacy for human drugs.

1. Procedures for restraint of the rabbits will be as follows:
   - Animals will be restrained in a rabbit restrainer (provided by ACS) or by using a clean towel with minimal restraint by an animal handler.
2. Procedure for Administration of Virus:
   - The rabbits will be restrained using a rabbit restrainer or with a towel and both hind flanks will be shaved.
   - The rabbits will be gently restrained with a towel and then injected intradermally with 100ul of buffer and the virus injected intradermally in one or two sites using a 25 gauge needle in one or both hind flanks.

3. Procedure for microchip placement:
   - A sterile Implantable Programmable Temperature Transponder chip (Biomedic Data Systems, Inc.) will be injected subcutaneously at the nape of the neck (dorsal cervical region). The chip transmits data for animal identification and body temperature.
   - The rabbits will be gently restrained with a towel.
   - The skin over the nape of the neck will be gently tented away from the back muscles.
   - The tented fur at the nape of the neck will be washed with ethanol.
   - A 2.2 by 14 mm transponder chip will be inserted under the skin by injection via the sterile 14 gauge needle that houses the chip.
   - The skin will be released and physically examined to ensure the chip is placed under the skin and that there is no damage to the skin.

4. Routine Physical Exam
   - A routine physical exam (abdominal palpitation, auscultation of the heart and lungs, assessment of attitude and hydration, etc.) will be performed daily.

5. Collection of Blood (Survival):
   - Rabbits are minimally restrained.
   - The animal is shaved and the skin is cleaned with 70% ethanol.
   - 1-2 ml of blood is collected from the lateral saphenous or ear vein with a 25 gauge needle every other day

6. Collection of Blood (Non-survival):
   - For collection of 10 ml blood, 50mg/kg ketamine and 10mg/kg xylazine will be injected into intramuscularly into the biceps femoris muscle or intraperitoneally.

- Blood will be collected by cardiac puncture using a sterile 22 gauge 1 ½ inch needle attached to a 12cc syringe.
- 100-150mg/kg body weight of pentobarbital will be injected into the heart after blood has been collected to induce euthanasia.

7. Necropsy and Tissue collection:
   - Upon euthanasia with pentobarbital and the confirmation of successful euthanasia, as determined by the absence of heart beat and respiration, a necropsy of the animal will be performed.
   - Tissue samples from all the organs, as well as lesions on the dermal phase of the animal, will be collected for virus isolation and characterization.

8. Administration of Imatinib
   - Imatinib will be administered orally (by feeding from a 1ml syringe) dissolved in sweetened water (10% glucose). Animals will receive 0-20 mg/kg of the drug at each dose, up to twice daily.

5. Provide scientific justification why pain and/or distress could not be relieved. State methods or means used to determine that pain and/or distress relief would interfere with test results.

Rabbit pox is a lethal infection in rabbits. Analgesics are not indicated in these studies because their affects would make it difficult to identify any adverse effects caused by drug treatment. Analgesics can have effects on respiration rate (a major criterion of euthanasia), temperature, heart rate and the general demeanor and behavior of the animal (attitude and posture). For example, personal communications with another RPV researcher at USAMRIID indicated they did not observe a temperature spike in infected animals, as they used analgesics every other day before drawing blood, and this suppressed the normally observed fever. It is critical to be able to identify any potential drug related toxicities during the course of these experiments.

Column E Explanation

This form is intended as an aid to completing the Column E explanation. It is not an official form and its use is voluntary. Names, addresses, protocols, veterinary care programs, and the like, are not required as part of an explanation. A Column E explanation must be written so as to be understood by lay persons as well as scientists.

1. Registration Number: 58-R-0003

2. Number 30 of animals used in this study

3. Species (common name) Rabbits of animals used in the study.

4. Explain the procedure producing pain and/or distress.

   Title: D290; Gene regulation of mammalian DNA viruses

   Please see attached for procedure information.

5. Provide scientific justification why pain and/or distress could not be relieved. State methods or means used to determine the pain and/or distress relief would interfere with test results. (For Federally mandated testing, see Item 6 below)

   Please see attached.

6. What, if any, federal regulations require this procedure? Cite the agency, the code of Federal Regulations (CFR) title number and the specific section number (e.g., APHIS, 9 CFR 113.102):

Agency _____ CFR _____

D290; *Gene regulation of mammalian DNA viruses*

4. Explain the procedure producing pain and/or distress, including reason(s) for species selected.

Rabbit pox is a lethal infection in rabbits. The differential pathogenesis caused by RPV and VV is not recapitulated in rodents, thus rabbits are the model system chosen. Analgesics are not indicated in these studies because their affects on the inflammatory and immune responses would hinder the distinctions observed in the two viral diseases. It is critical that the rabbit pox and vaccinia viral diseases be distinguishable. For the screening of recombinant viruses it is also critical for the disease pathways be uninhibited for the detection of virulent viruses.

1. Procedures for restraint of the rabbits will be as follows:
   - Animals will be restrained in a rabbit restrainer (provided by ACS) or by using a clean towel with minimal restraint by an animal handler.
   - The rabbits will be restrained using a rabbit restrainer or with a towel and both hind flanks will be shaved.
   - The rabbits will be gently restrained with a towel and then injected interdermally with 100ul of buffer and the virus injected intradermally in one or two sites using a 25 gauge needle in one or both hind flanks concurrently in Experiment 1 and 3, with animals in Experiment 2 receiving 100ul of buffer and the virus injected intradermally in one to five sites concurrently using a 25 gauge needle in one or both hind flanks.
   - A sterile Implantable Programmable Temperature Transponder chip (Biomedic Data Systems, Inc.) will be injected subcutaneously at the nape of the neck (dorsal cervical region). The chip transmits data for animal identification and body temperature.
   - The rabbits will be gently restrained with a towel.
   - The skin over the nape of the neck will be gently tented away from the back muscles.
   - The tented fur at the nape of the neck will be washed with ethanol.
   - A 2.2 by 14 mm transponder chip will be inserted under the skin by injection via the sterile 14 gauge needle that houses the chip.
   - The skin will be released and physically examined to ensure the chip is placed under the skin and that there is no damage to the skin.
   - A routine physical exam (abdominal palpitation, auscultation of the heart and lungs, assessment of attitude and hydration, etc.) will be performed daily.

2. Collection of Blood (Survival):
   - Rabbits are minimally restrained.
   - The animal is shaved and the skin is cleaned with 70% ethanol.
   - 1-2ml of blood is collected from the lateral saphenous vein with a 25 gauge needle every 2-3 days after virus administration
   - For collection of 10ml blood, 50mg/kg ketamine and 10mg/kg xylazine will be injected into intramuscularly into the biceps femoris muscle or intraperitoneally.
   - Blood will be collected by cardiac puncture using a sterile 22 gauge 1 ½ inch needle attached to a 12cc syringe.
   - 100-150mg/kg body weight of pentobarbital will be injected into the heart after blood has been collected to induce euthanasia.

3. Necropsy and Tissue collection:
    - Upon euthanasia with pentobarbital and the confirmation of successful euthanasia, as determined by the absence of heart beat and respiration, a necropsy of the animal will be performed.
    - Tissue samples from all the organs, as well as lesions on the dermal phase of the animal, will be collected for virus isolation and characterization.

5. Provide scientific justification why pain and/or distress could not be relieved. State methods or means used to determine that pain and/or distress relief would interfere with test results.

Rabbitpox is a lethal infection in rabbits. The differential pathogenesis caused by RPV and VV is not recapitulated in rodents, thus rabbits are the model system chosen. Analgesics are not indicated in these studies because their affects on the inflammatory and immune responses would hinder the distinctions observed in the two viral diseases.

Column E Explanation

This form is intended as an aid to completing the Column E explanation. It is not an official form and its use is voluntary. Names, addresses, protocols, veterinary care programs, and the like, are not required as part of an explanation. A Column E explanation must be written so as to be understood by lay persons as well as scientists.

1. Registration Number: 58-R-0003

2. Number 18 of animals used in this study

3. Species (common name) Rabbits of animals used in the study.

4. Explain the procedure producing pain and/or distress.

   Title: D968; Antiviral activity of lipid-conjugated nucleoside analogs against orthopoxviruses

   Please see attached for procedure information.

5. Provide scientific justification why pain and/or distress could not be relieved. State methods or means used to determine the pain and/or distress relief would interfere with test results. (For Federally mandated testing, see Item 6 below)

   Please see attached.

6. What, if any, federal regulations require this procedure? Cite the agency, the code of Federal Regulations (CFR) title number and the specific section number (e.g., APHIS, 9 CFR 113.102):

Agency _____ CFR _____

D968; *Antiviral activity of lipid-conjugated nucleoside analogs against orthopoxviruses*

4. Explain the procedure producing pain and/or distress, including reason(s) for species selected.

Rabbits infected with rabbit pox virus are a unique animal model for smallpox studies because the disease closely mimics the pathology of the human disease, and because rabbit pox virus can be spread between animals in the absence of mechanical or insect vectors. In addition, federal regulations require the use of 2 animal species when using animal data to establish efficacy for human drugs.

1. Procedures for restraint of the rabbits will be as follows:
    - Animals will be restrained in a rabbit restrainer (provided by ACS) or by using a clean towel with minimal restraint by an animal handler.
    - The rabbits will be restrained using a rabbit restrainer or with a towel and both hind flanks will be shaved.
    - The rabbits will be gently restrained with a towel and then injected interdermally with 100ul of buffer and the virus injected intradermally in one or two sites using a 25 gauge needle in one or both hind flanks concurrently in Experiment 1 and 3, with animals in Experiment 2 receiving 100ul of buffer and the virus injected intradermally in one to five sites concurrently using a 25 gauge needle in one or both hind flanks.
    - This procedure does not cause excessive pain to the animal as the chip is placed just under the skin. The benefits of placing the chips into the animals and having them experience a momentary needle stick is far less stress than the alternative which is a rectal temperature taken multiple times within a day.
    - A sterile Implantable Programmable Temperature Transponder chip (Biomedic Data Systems, Inc.) will be injected subcutaneously at the nape of the neck (dorsal cervical region). The chip transmits data for animal identification and body temperature.
    - The rabbits will be gently restrained with a towel.
    - The skin over the nape of the neck will be gently tented away from the back muscles.
    - The tented fur at the nape of the neck will be washed with ethanol.
    - A 2.2 by 14 mm transponder chip will be inserted under the skin by injection via the sterile 14 gauge needle that houses the chip.
    - The skin will be released and physically examined to ensure the chip is placed under the skin and that there is no damage to the skin.
    - A routine physical exam (abdominal palpitation, auscultation of the heart and lungs, assessment of attitude and hydration, etc.) will be performed daily.

2. Collection of Blood (Survival):
    - Rabbits are minimally restrained.
    - Approximately an area of 4 inches by 4 inches of the hind flank of the animal is shaved and the skin is cleaned with 70% ethanol.
    - 1-2ml of blood is collected from the lateral saphenous or ear vein with a 25 gauge needle daily

- Kidney parameters will not be tested as CDV has not been observed to be nephrotoxic at the dose that is proposed. There has been no nephrotoxocity observed in animals (rabbits as well as mice) given LIP-CDV.
- For collection of 10ml blood, 50mg/kg ketamine and 10mg/kg xylazine will be injected intramuscularly into the biceps femoris muscle or intraperitoneally.
- Blood will be collected by cardiac puncture using a sterile 22 gauge 1 ½ inch needle attached to a 12cc syringe.
- 100-150mg/kg body weight of pentobarbital will be injected into the heart after blood has been collected to induce euthanasia.

3. Necropsy and Tissue collection:
   - Upon euthanasia with pentobarbital and the confirmation of successful euthanasia, as determined by the absence of heart beat and respiration, a necropsy of the animal will be performed.
   - Tissue samples from all the organs, as well as lesions on the dermal phase of the animal, will be collected for virus isolation and characterization.

4. Administration of Cidofovir
   - Cidofovir will be administered in an ear vein dosed at 5-20 mg/kg.

5. Administration of LIP-CDV
   - The oral LIP-CDV will be administered in a liquid form sweetened with sugar water at a dose of 5-20 mg/kg/day with the animals receiving a total volume of no more than 10ml at a given dosing event.

5. Provide scientific justification why pain and/or distress could not be relieved. State methods or means used to determine that pain and/or distress relief would interfere with test results.

Rabbitpox is a lethal infection in rabbits. Analgesics are not indicated in these studies because their affects would make it difficult to identify any adverse effects caused by drug treatment. It is critical to be able to identify any potential drug related toxicities during the course of these experiments. Analgesics are not indicated in these studies because of their affects on the inflammatory and immune responses that could possibly hinder the typical clinical observations previously documented in rabbits infected with RPV. It is critical that the rabbitpox viral disease be observed unhindered.